REMARKS

Amendments to the Claims

As indicated above, Applicants have amended Claim 1 to incorporate the limitation of dependent Claim 82, which specifies that the membrane in the process of Claim 1 has pores that have a diameter of 0.001 μm to 50 μm. In addition to Claim 82, support for the amendment is also found in the specification at page 9, lines 21-22. Applicants have therefore also canceled Claim 82 to eliminate redundancy in the claims. In addition, Applicants have adjusted the dependencies of Claims 90 and 91 so that these claims now depend from Claim 1, as amended herein, instead of the now canceled Claim 82. The amendments add no new matter.

Applicants take this opportunity to note that cancellation of any claim in this application should not be interpreted as an abandonment of the subject matter of such canceled claim or of any subject matter described in the specification. Applicants reserve the right to pursue coverage for the subject matter of any canceled claim(s) or for any subject matter described in the specification in a continuation or divisional application.

Applicants have also added new dependent Claims 92, 93, 94, 95, 96, 97, 98, 99, and 100 to cover specific embodiments of the process of amended Claim 1 wherein the membrane has pores with a diameter of at least 0.2 μm, at least 0.45 μm, at least 0.65 μm, at least 1 μm, at least 1.2 μm, at least 3 μm, at least 5 μm, at least 10 μm, and at least 20 μm, respectively. Support for new Claims 92-100 is found in the specification. For example, for new Claims 92 (0.2 μm), 93 (0.45 μm), 94 (0.65 μm), 96 (1.2 μm), 98 (3 μm), 99 (5 μm), and 100 (20 μm), see Table 5 in Example 5 at page 25 of Applicants' specification. For new Claim 95 (1 μm), see, for example, last row of Table 13, page 37, of the specification. For new Claim 97 (3 μm), see, for example, second row of Table 3, page 21, of the specification. Accordingly, new dependent Claims 92-100 add no new matter.

Entry of the amendments to the claims is respectfully requested.

Clarification of Claims 65 and 66 (not entered)

Applicants now address the Examiner's comments made in the section entitled "Miscellaneous" on page 11 of the Office Action, dated August 8, 2007, in which the Examiner stated that the status indicator for Claims 65 and 66, i.e., "not entered", is improper and that clarification on this issue is required. Applicants respectfully note that all of the documents issued by the Patent Office and submitted by Applicants are of record regarding Claims 65 and 66. Nevertheless, Applicants provide the following review as instructed by the Examiner in the pending Office Action:

First, "not entered" is a proper status indicator according to 37 CFR § 1.121(c) and as demonstrated in the Patent Office's own Sample Format for Revised Amendment Practice (posted 30 June

2003) from the USPTO website. Applicants sought entry of new Claims 65 and 66 in Applicants' Response to an Office Action under 37 CFR § 1.116, submitted June 30, 2003. The claims were not entered according to the Advisory Action, dated July 15, 2003. In Applicants' subsequent new Listing of the Claims in a Response filed concurrently with a Request for Continued Examination (RCE) pursuant to 37 CFR § 1.114, dated October 21, 2003, Applicants designated Claims 65 and 66 as "not entered" and did **not** reproduce the text of these claims in accordance with the Patent Office's own Sample Format for Revised Amendment Practice. Nearly two years later, Legal Instruments Examiner Tina Plunkett issued a Notice of Non-Compliant Amendment (37 CFR § 121), dated September 30, 2005, in which Examiner Plunkett stated that Applicants' Amendment, dated June 22, 2005, (which was used as a submission for Applicants' second RCE, filed September 15, 2005) was non-compliant because "Claims 65 and 66 were not listed". See, copy of Notice of Non-Compliant Amendment, attached at **Tab A**. The undersigned Applicants' attorney recalls a telephonic conversation **in which Examiner Plunkett specifically mentioned that the text of the "not entered" Claims 65 and 66 must be provided to overcome the Notice of Non-Compliant Amendment**. Accordingly, Applicants submitted a Response to Notice of Non-Compliant Amendment on October 7, 2005, in which Applicants included the text of "not entered" Claims 65 and 66. See, copy of Applicants' Response to Notice of Non-Compliant Amendment, attached at **Tab B**; particularly, Remarks at page 13.

Applicants now conclude that Examiner Plunkett's instructions that Applicants must reproduce the text of Claims 65 and 66 to overcome the Notice of Non-Compliant Amendment is clearly **not** proper with the current form of 37 CFR § 1.121(c)(4) and the Patent Office's own Sample Format for Revised Amendment Practice. Accordingly, as noted above, Applicants have in the above Listing of the Claims eliminated the text of Claims 65 and 66 and simply recited the claim numbers with the proper designation "not entered". Entry of the amendments is respectfully requested.

The above review of this record, the citations to regulations and the Patent Office's Sample Format, and the attached documents from the record show that Applicants have always sought to comply with the regulations and instructions from the Patent Office with respect to Claims 65 and 66 and that the current designation and presentation of Claims 65 and 66 as "not entered" are now in compliance with 37 CFR § 1.121(c)(4). Accordingly, Applicants respectfully request that the Examiner deem Claims 65 and 66 in compliance with 37 CFR § 1.121(c).

In the event that the current file for this application in the Patent Office is missing any documents, Applicants will be happy to provide replacement copies from their file upon request.

Rejections Under 37 CFR § 102 and § 103

In the Office Action, dated August 8, 2007, the Examiner rejected Claims 1, 3-5, 24, 25, 33, 34, 36, 51, 69, 72, 76-80, 82, 90, and 91 under 37 CFR § 102 as anticipated by European patent publication No. 0 431 905 A1 ("Ogawa"). The Examiner also rejected Claims 9-14, 39, 40, 55, 59-61, 70-72, 77, 78, and 89 under 37 CFR § 103 as obvious over the primary reference Ogawa in view of U.S. Patent No. 5,234,824 ("Mullis"). The Examiner also rejected Claims 9-17, 22, 26-31, 35, 37-40, 53-55, 59-64, 70, 71, 73, 74, 81, and 83-89 under 37 CFR § 103 as obvious over the primary reference Ogawa in view of Mullis, Pfister (*J. Biol. Chem., 271(3)*: 1687-1694 (1996), U.S. Patent No. 5,234,809 ("Boom"), U.S. Patent No. 6,383,393 ("Colpan"), and U.S. Patent No. 5,985,572 ("Macfarlane"). Applicants respectfully traverse these rejections.

Clarifications of the Primary Reference Ogawa

Before addressing the three sets of rejections, Applicants provide the following comments to review and to clarify important facts regarding the primary reference Ogawa and Applicants' claimed invention.

First, Applicants note that their previous response and the declaration by co-inventor Uwe Oelmüller, submitted May 18, 2007, were properly provided to explain the facts of and differences between Applicants' claimed process and the prior art methodologies of the primary reference Ogawa and other cited references (see, e.g., paragraph 4, Dr. Oelmüller's declaration). As explained in their prior response and Dr. Oelmüller's declaration, Ogawa expressly and clearly teaches isolation of phage DNA using ultrafiltration, which is a well known biochemical technique that separates molecules on the basis of size, i.e., a mixture of differently sized molecules are separated on the basis of whether or not particular molecules are sufficiently small to pass through the pores of an ultrafiltration membrane (ultrafilter) or sufficiently large not to pass through the pores of the ultrafilter. Ogawa describes the use of ultrafiltration to isolate phage DNA on one side of an ultrafilter having pores whose size is both sufficiently small to retain the phage DNA molecules but sufficiently large so as to allow other smaller molecules, such as denatured phage proteins, to pass through to the other side of the ultrafilter, thereby purifying the phage DNA retained on the ultrafilter (in effect, *straining* the solution through an ultrafilter to "catch" the phage DNA).

In contrast to Ogawa's use of ultrafiltration to retain nucleic acid molecules that are larger than the pore size of a particular ultrafiltration membrane, Applicants' process involves charging one side of a non-siliceous membrane and immobilizing the nucleic acids to that side of the non-siliceous membrane by **binding** the nucleic acid molecules to the one side of a non-siliceous membrane in the presence of an immobilization buffer. The bound nucleic acid is subsequently eluted (released) and retrieved from the

same side on which it was bound. See, e.g., Claim 1 as amended herein. Contaminating proteins and other non-nucleic acid molecules are not so bound to the non-siliceous membrane and therefore can pass through the pores to the other side of the membrane, thereby purifying the nucleic acid that remains bound to the side of the membrane to which the nucleic acids were initially charged. As discussed more fully below, the fact that Applicants' claimed process involves an actual binding of nucleic acids to one side of a non-siliceous membrane and not simply retention on the membrane of nucleic acid molecules that are larger than the membrane's pore size is clearly shown by the fact that excellent yields of purified nucleic acid molecules can be isolated according to Applicants' process even when the non-siliceous membrane has a pore size that is clearly larger than the size of the isolated nucleic acid molecules.

Applicants affirm the relevance of and facts explained in their prior response and the declaration of co-inventor, Dr. Uwe Oelmüller, submitted May 18, 2007.

Clarification of the Membrane Filters in Ogawa

In the Office Action, the Examiner characterized Ogawa as describing a process for isolating nucleic acids that comprises charging one side of a non-siliceous membrane that may have pore sizes from 0.005 μm to 0.45 μm and immobilizing the nucleic acids on one side of the membrane in the presence of an immobilization buffer. This is not an accurate description of Ogawa. In Ogawa, different membranes are used for different reasons, but only one is an ultrafiltration membrane, i.e., an ultrafilter, that is employed for retaining naked phage DNA molecules on its surface that are too large to pass through the pores of the ultrafilter, while smaller molecules, such as proteinase K and decomposed phage proteins, freely pass through the pores to other side of the ultrafilter. At no place does the process in Ogawa employ an actual binding of the naked phage DNA to ultrafilter as taught by Applicants. This is clearly seen in the Example of Ogawa. First, a bacterial cell culture medium used to grow up M13 phage particles is pre-filtered through a membrane having a pore size of 0.45 μm (see, col. 4, lines 20-22, of Ogawa) to remove cells and cell debris. As noted in Applicants' prior response and Dr. Oelmüller's declaration, submitted May 18, 2007, this step in Ogawa is a standard filtration step used to filter out cells and cell debris out from a culture medium and typically employs membranes having a pore of size of 0.22 μm or, as in Ogawa's Example, 0.45 μm, both of which pore sizes are sufficiently small to retain bacterial cells and cell debris and sufficiently large to allow the much smaller phage particles to easily pass through with the clarified liquid medium. (The use of a membrane to remove microorganism cells, not to retain DNA, is also specifically recited in Claim 1 of Ogawa, and the embodiment wherein such a membrane has a pore size of 0.22 μm or 0.45 μm is recited in dependent Claim 2 of Ogawa.) In the Example of Ogawa, the filtered medium containing the suspended phage particles is then applied to an actual ultrafiltration membrane (ultrafilter) that has a fractionation molecular weight of 300,000 daltons in order

to remove low molecular weight components in the culture medium. The phage particles are retained on the ultrafilter and then decomposed with proteinase K to release the naked phage DNA. The proteinase K and decomposed phage capsule proteins are then washed through the pores of the ultrafilter, leaving the naked phage DNA that is larger than the pore size of the ultrafilter retained on the ultrafilter (see, col. 4, lines 23-39, of Ogawa).

As noted in Applicants' prior response and in Dr. Oelmüller's declaration, submitted May 18, 2007, an ultrafilter having a fractionation molecular weight of 300,000 daltons should have a pore size in the range of 0.035 μm and thus would be expected to retain the larger phage DNA and allow smaller molecules, such as the proteinase K and the decomposed phage proteins, to be easily washed through and away from the retained naked phage DNA. Ogawa also mentions that a preferred ultrafiltration membrane useful in the process has a fractionation molecular weight of 20,000 daltons to 1,000,000 daltons and notes that if the fractionation molecular weight is smaller than this range, then the efficiency of the removal of the decomposed proteins is reduced, **while if the fractionation molecular weight of the ultrafilter is larger than this range, the phage DNA may pass through the ultrafilter** (see, col. 3, lines 31-38, of Ogawa). This clearly shows that Ogawa describes a prior art methodology for purifying DNA molecules based on their size relative to the pore dimensions of an ultrafiltration membrane so that a DNA molecule is retained on one side of a selected ultrafiltration membrane due to the fact that its size is larger than the dimensions of the pores of the membrane. Thus, the DNA molecule or any other comparably sized molecule is excluded from passing through the pores to the other side of an appropriately selected ultrafiltration membrane. There is no teaching or suggestion in Ogawa to actually bind the phage DNA to a membrane as in Applicants' claimed process, and the ultrafiltration methodology of Ogawa cannot isolate a DNA molecule using a membrane whose pore dimensions are larger than the desired DNA molecule. Ogawa therefore does NOT teach "immobilizing the nucleic acids. . .by binding the nucleic acids to said one side of the membrane" as required by Applicants' Claim 1; rather, Ogawa teaches filtration, i.e., immobilizing nucleic acids (or other materials) by straining against a membrane on the basis of size. Applicants' point is that the recitation of immobilization by binding to a non-siliceous membrane is the distinction of the present invention over filtration methods taught by Ogawa , and that the advantage of binding makes the success of the method independent of membrane pore size.

As previously noted in Applicants' response and Dr. Oelmüller's declaration, submitted May 18, 2007, persons skilled in the art are aware that ultrafiltration membranes used in separating biomolecules have pore sizes in the range of 0.001 μm to 0.100 μm. Membrane pore sizes greater than 0.100 μm are sufficiently large to permit most individual molecules, including most DNA, RNA, and protein

molecules, to pass through the membrane and are not classified as ultrafiltration membranes. See, e.g., paragraph bridging pages 4 and 5 of Applicants' May 18, 2007 Response; paragraphs 9-15 and Tab C of the Oelmüller declaration submitted May 18, 2007. In contrast, as shown in Table 5 of Example 5 at page 25 of Applicants' specification, total RNA was isolated from HeLa cells using non-siliceous membranes having pore sizes ranging from 0.01 μm to 20 μm. Persons skilled in the art would appreciate that the range of pore sizes of the non-siliceous membranes in Table 5 of Example 5 in Applicants' specification clearly crosses over and exceeds the upper limit of the pore size (0.1 μm) of any ultrafiltration membrane, that pore sizes greater than 0.1 μm are clearly larger than the size of the RNA molecules isolated, and, therefore, that the data in Table 5 show that nucleic acid molecules are clearly being preferentially **bound** to the membrane and not simply retained by having a size larger than the pores of the membrane. Otherwise, in the absence of actually being bound to the membranes, the RNA molecules would not have been isolated but would have easily passed through the membranes with pore sizes of 0.2 μm, 0.45 μm, 0.65 μm, 1.2 μm, 5 μm, 10 μm, and 20 μm. In fact, as shown in Table 5, noticeably high yields of purified RNA were obtained using membranes that had such pores sizes that range from two times (0.2 μm) to 200 times (20 μm) larger than the upper pore size limit of 0.100 μm used in ultrafiltration. As mentioned above, Ogawa used an ultrafilter with pore diameter of 0.035 μm to retain the larger naked M13 phage DNA. Note also that an entire cell of the bacterium *Escherichia coli* is typically about 1.0 μm wide and 2.0 μm or more long (see, paragraph 9 of the Oelmüller declaration submitted May 18, 2007) and, thus, such a cell could easily pass through the pores of several of the membranes listed in Table 5. Thus, as Applicants' specification and the data therein make clear, Applicants' claimed process of isolating nucleic acids employs actual binding of the nucleic acid molecules to one side of a non-siliceous membrane and subsequent elution and retrieval thereform and does not rely on mechanical retention of nucleic acid molecules by the membrane as in the prior art methodology of Ogawa.

Rejections Under 35 USC § 102

With respect to the rejection of Claims 1, 3-5, 24, 25, 33, 34, 36, 51, 69, 72, 76-80, 82, 90, and 91, as anticipated by Ogawa, Applicants respectfully traverse for the reasons discussed herein.

For anticipation under 35 USC § 102 by a printed publication, that publication must teach each and every element or aspect of the claimed invention. As explained in MPEP § 2131:

> **"TO ANTICIPATE A CLAIM, THE REFERENCE MUST TEACH EVERY ELEMENT OF THE CLAIM**
>
> " 'A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.' *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). 'The identical

> invention must be shown in as complete detail as is contained in the . . . claim.' *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989)." (emphasis in original).

As noted above, Applicants have amended Claim 1 to incorporate the limitation that the non-siliceous membrane has the range of pore size recited in original Claim 82 (now canceled). As also explained above, ultrafiltration employed by Ogawa only retains nucleic acid molecules on one side of a membrane if the nucleic acid molecules are larger than the pore size of the selected ultrafiltration membrane. In contrast, as illustrated by actual data in Applicants' specification (e.g., Table 5, Example 5), Applicants' claimed process comprises immobilizing nucleic acids to one side of a non-siliceous by binding the nucleic acids to that side of the membrane in the presence of an immobilization buffer. Thus, the prior art methodology of Ogawa is not the same as Applicants' claimed process, and Ogawa clearly does not teach each and every element of Applicants' claims as amended herein. Accordingly, since Ogawa does not qualify as a reference to anticipate the claims under 35 USC § 102, the Examiner is respectfully requested to reconsider and withdraw the rejections.

Rejection Under 35 USC § 103 (Ogawa in view of Mullis)

The Examiner also rejected Claims 9-14, 39, 40, 55, 59-61, 70-72, 77,78, and 89 under 35 USC § 103 as obvious over Ogawa in view of Mullis. Applicants respectfully traverse for the reasons explained below.

In the rejection, the Examiner stated:

> "**Ogawa et al. teaches the essential features of the claimed invention.** . . . **However, Ogawa et al. does not explicitly teach the standard components of the immobilization and washing solutions which are routinely used to prepare nucleic acid** containing compositions for immobilization on membranes and wash the filters containing the immobilized nucleic acids or the use of hydrophilic membranes or the chemical treatments of nucleic acids between the time the nucleic acids are released from the membrane and the time they are collected." (page 5, Office Action of August 8, 2007; emphasis added).

As noted above, the ultrafiltration methodology of Ogawa clearly does not teach an essential feature of Applicants' process, i.e., immobilizing nucleic acids on one side of a membrane by binding the nucleic acids to that side of the membrane in the presence of an immobilization buffer. Thus, the opening assertion of the Examiner, that Ogawa "teaches the essential features" of the invention, is incorrect.

Continuing on this false premise, the Examiner asserts that Mullis is able to provide a description of various immobilization and washing solutions used to isolate DNA so that the combination of Ogawa and Mullis renders obvious those claims of Applicants' that are directed to specific ingredients for immobilization buffers and washing solutions. The Examiner references with particularity the Abstract,

the last paragraph in column 2, columns 5-6, Examples 6-8, and Claims 1-12 of Mullis. See, pages 6-7, of the Office Action of August 8, 2007.

As already explained, Ogawa expressly teaches a prior art method of isolating phage DNA using ultrafiltration to mechanically separate on the basis of size (as opposed to separation by binding as claimed herein). Similarly, though not identically, the prior art methodology of Mullis is directed specifically to obtaining a sufficiently high molecular weight form of genomic DNA from gently lysed cells that can then be physically retained or trapped on or in a membrane and prevented from passing through the dimensions of the membrane's pores:

> "The method involves **gently lysing** the membranes of the cells to yield a lysate containing **genomic DNA in a high molecular weight form**. The lysate is moved through a porous filter **to selectively trap the high molecular weight DNA on the filter**." (Abstract, of Mullis; emphasis added).
>
> "The method comprises a first step of gently lysing the membranes of the cells in a blood sample. . . . **An object of gently lysing is to avoid high shear forces on the DNA so as to yield a portion of the DNA having a sufficiently high molecular weight to be selectively trapped on a membrane filter**." (col. 2, lines 41-49, of Mullis; emphasis added).
>
> "The invention embodies lysing that is sufficiently gentle so as to produce a lysate containing **a portion of DNA having a molecular weight that allows it to be selectively trapped on a porous filter** when the lysate is filtered through the filter." (col. 5, lines 1-5, of Mullis; emphasis added).
>
> ". . . [I]t is believed that high molecular weight DNA released by gentle lysis of cells is trapped on the porous filter by virtue of the fact that the DNA chains are considerably longer than the inter-pore distance on the surface of the filter such that separate regions of a single high molecular weight DNA chain may be simultaneously drawn into different pores, thus preventing complete passage of the molecule through either poor. **Accordingly, the high molecular weight DNA molecule is effectively trapped on and/or in the filter**." (col. 6, lines 17-27, of Mullis; emphasis added).
>
> "As in Example 1, above, heparinized, human whole blood was collected, gently lysed, and transferred to a centrifuge filter unit. The centrifuge filter unit **served as the filter for retaining the high molecular weight DNA**." (col. 8, lines 50-54, of Mullis; emphasis added).

See, also Claims 1 and 2, of Mullis.

The above excerpts clearly show that Mullis describes another example of the prior art methodology that is based on a **physical retention or entrapment** on or in a membrane of a DNA species that is larger than the pore dimensions of the membrane. Contrary to the Examiner's characterization of Mullis, the various salts, detergents, and other compounds in Mullis are not used to prepare immobilization buffers to effect binding of any nucleic acid to a membrane as in Applicants' claimed process, but rather as components of lysis buffers that can be employed to gently lyse cells in a manner that reduces shear forces on the genomic DNA and thereby obtain a portion of the genomic DNA that has a sufficiently high molecular weight to be entrapped in or on a membrane filter (see, e.g., in Mullis, col. 7, lines 51-58 (Example 1); col. 9, lines 34-39 (Example 3)). Thus, both Ogawa and Mullis, individually and together, provide a description of prior art methodologies that teach how to release specific DNA molecules that are sufficiently larger than the particular pore dimensions of a selected membrane so that those DNA molecules can be retained or trapped on the selected membrane.

It would be clear to persons of ordinary skill in the art that neither Ogawa nor Mullis, alone or in combination, teaches or suggests the essential features of Applicants' claimed invention wherein nucleic acids are bound to one side of a membrane in the presence of an immobilization buffer. Accordingly, it is clear error to state that the ultrafiltration process of Ogawa teaches Applicants' claimed process and that the combination of Ogawa and specific size exclusion methodology of Mullis renders obvious additional embodiments of Applicants' claimed process when Applicants' claimed process that employs immobilization buffers to effect actual binding of nucleic acids to a non-siliceous membrane or washing solutions to wash nucleic acid molecules so bound to a membrane.

Applicants agree that Mullis mentions that in a preferred embodiment, the high molecular weight genomic DNA released using various gentle lysis solutions is trapped on membranes with pore sizes ranging from 0.2 μm to 0.8 μm (col. 5, lines 42-46, of Mullis) and that Mullis specifies that a most preferable filter has a pore size of about 0.45 μm. Again, however, the fact that such membranes existed in the prior art and that they were used in the prior art retention methodology of Mullis to retain a particular portion of high molecular weight genomic DNA that is larger than such pore dimensions is not a teaching of or suggestion to practice Applicants' claimed process, in which nucleic acids are actually bound to a membrane in the presence of an immobilization buffer such that the nucleic acids may be isolated using a membrane having a pore size ranging from size of 0.001 μm to 50 μm and that such bound nucleic acids may also be retrieved from the same side of the membrane to which they were bound. Thus, the Examiner has incorrectly characterized the prior art methodologies in Ogawa and Mullis and what a combination of these references provided to the prior art in the absence of Applicants' claimed invention.

Finally, the Examiner asserts that Mullis in combination with Ogawa provides an example of the embodiment of Applicants' invention recited in pending Claim 9 in which between release and removal of the isolated nucleic acids, at least one chemical reaction is carried out on the nucleic acids. In particular, the Examiner states:

> ". . . Mullis teaches that the nucleic acids can be subject to restriction endonuclease digestion **after release** from the membranes to determine the levels of release and recovery of the nucleic acids from the filters. . . . The ordinary skilled artisan would have been motivated to combine the teachings of Ogawa et al. on the purification of phage nucleic acids with the teachings of Mullis on the use of standard nucleic acid immobilization and washing solutions for use with hydrophobic or hydrophilic membranes because Mullis teaches that said solutions can be routinely used in processes for purifying nucleic acids on membranes. The ordinary skilled artisan would have been motivated to digest the nucleic acids released from the filters in order to measure the level (the purity) of recovery of the nucleic acids using various modifications of the immobilization and washing techniques." (Page 6, of the Office Action of August 8, 2008; emphasis added).

For the record, Applicants have not found and the Examiner has not indicated where Mullis provides a description of using restriction endonuclease digestion to test the purity of high molecular weight genomic DNA **after a release** from a membrane. If the Examiner is referring to Example 7 of Mullis (and other references in Mullis thereto), then the Examiner's characterization is clear error. Example 7 is one of several examples in which Mullis attempts to release the high molecular weight genomic DNA entrapped in a membrane. In Example 7, the high molecular weight DNA entrapped in the membrane was subjected to restriction endonuclease digestion **in an effort to release the DNA trapped in the membrane**, not to test its purity after release from a membrane. Mullis specifically states that the purpose of Example 7 was to determine the effects of restriction endonuclease digestion, heat (as in Examples 6), and microwave radiation on the release of the entrapped DNA from the filter (col. 13, lines 19-22, in Example 7 of Mullis). Mullis summarized the findings as follows:

> "The results indicated that the concentration of restriction endonuclease used in this Example released some DNA trapped on the filter. However, the release of DNA was not as rapid as the release due to microwave radiation." (col. 13, line 64-col. 14, line

In addition, the use of a restriction endonuclease digestion to release entrapped DNA from a membrane may be of technical interest to Mullis, but it would be of little utility to a person of ordinary skill in the art who desires to purify intact genomic DNA without then resorting to a digestion that destroys it. Claim 9 covers the embodiment of the process of Claim 1 as amended herein wherein

between the release and removal steps at least one chemical reaction is carried out on the nucleic acids. "Between" clearly indicates **after** release and **before** removal of the nucleic acid molecules from the membrane, **not** as in Mullis for the purpose of releasing some portion of the nucleic acids.

Accordingly, Mullis does not provide a teaching that in combination with Ogawa teaches or suggests the embodiment of Applicants' process recited in dependent Claim 9 in which a nucleic acid molecule that is isolated **according to Claim 1** is also subjected to at least one chemical reaction between the release and removal steps.

Although there is no teaching or suggestion in either of Ogawa or Mullis that the two references be combined to provide Applicants' claimed process, the above detailed explanation clearly shows that even if the prior art methodologies of Ogawa and Mullis are combined, the combination does not provide the prior art with Applicants' distinctly different process, including any of the embodiments of the process specified in dependent Claims 9-14, 39, 40, 55, 59-61, 70-72, 77, 78, and 89. Accordingly, Applicants submit that it is clear that the combination of Ogawa and Mullis does not render Applicants' claims as amended herein obvious under 35 USC § 103, and the Examiner is respectfully requested to reconsider and withdraw the rejections.

Rejections Under 35 USC § 103 (Ogawa in view of Mullis, Pfister, Boom, Colpan, and Macfarlane)

In the Office Action, the Examiner rejected Claims 9-17, 22, 26-31, 35, 37-40, 53-55, 59-64, 70, 71, 73, 74, 81, and 83-89 under 35 USC § 103 as obvious over Ogawa in view of Mullis, Pfister, Boom, Colpan, and Macfarlane.

The Examiner applies Ogawa in view of Mullis as for the above-mentioned rejection. The Examiner states that Pfister and Boom are applied as previously stated in the Office Action of August 23, 2006. In particular, the Examiner notes in the pending Office Action that Pfister was cited to demonstrate the use of standard solutions in lysing cells and immobilizing nucleic acids on filters (page 10, Office Action of August 8, 2007). With respect to Boom as well as its combination with Ogawa and Pfister, the previous Office Action of August 23, 2006 stated:

> "As detailed previously, Boom teaches methods of purifying nucleic acids (e.g., RNA, dsDNA, ssDNA). (col. 8, ll. 15) and that . . . various surfaces, including siliceous and non-siliceous (e.g., silica derivatives, latex, PVDF, nitrocellulose, Hybond-N), can be utilized in purifying nucleic acids. (e.g., col. 6, ll. 5-27). Applicant's specific citation of one example of the many examples present in the [Boom] reference does not alter the salient teachings of the reference, that nucleic acids may be purified using various buffers (immobilization and elution) and the use of various binding substrates.

> "**The question at hand is whether a person of skill, reading Ogawa would have the motivation to combine that which was known in the art with the teachings of Pfister and [Boom] to devise the instant claimed inventions. Fully considering what is taught by the references and [what] would have been [known] in the art, it is still deemed that combined teachings of Ogawa, Pfister, and [Boom] make obvious the instant claimed inventions.** A person of skill in the art would have been motivated to combine the teachings [of the references to] optimize the buffer/membrane combinations, depending on the species of nucleic acids sought to be purified, and to conduct routine experimentation to obtain optimum conditions for purifying a particular species of nucleic acid of the instant claimed methods." (pages 8-9, Office Action of August 23, 2006; emphasis added)

Now in the pending Office Action of August 8, 2007, the Examiner states that Colpan teaches the use of chaotropic agents (such as guanidinium isothiocyanate, potassium iodide, sodium perchlorate, etc.) at levels of 1-8 molar in the immobilization buffer as well as use of alcohols and phenol in the immobilization buffer. See, page 7, of the Office Action. The Examiner also cites Macfarlane for teaching use of salts of oxalic, malonic, citric, or succinic acid in nucleic acid purification. See, page 8, of the Office Action.

For convenience in explaining Applicants' reasons to traverse this rejection, the Examiner's reasoning in the current Office Action for why the combination of Ogawa in of view Mullis, Pfister, Boom, Colpan, and Macfarlane renders the claims obvious is provided below:

> "**The essential features of applicant's invention are taught by Ogawa et al. (see above 102(b) rejection). The remaining limitations relate to the standard procedures and components used in nucleic acid purification techniques** (i.e. the types of membranes used, the standard solutions (immobilization buffers) used to solubilize compositions comprising the nucleic acids of interest as well as the agents used in said solutions and in washing buffers to wash the membranes after the nucleic acids are applied. All of the components of the immobilization solutions claimed by applicants, such as chaotropic agents including guanidinium isothiocyanate, potassium perchlorate, etc., as well as agents such as alcohols, phenols, salts of organic dicarboxylic acids (i.e., citric acid, oxalic acid, etc.) and the agents used in washing solutions (i.e. containing an alcohol, phenol, etc.) are standard components in procedures for isolating nucleic acids (as evidenced, for example, by the teachings of Colpan et al. and Macfarlane[])]. **The prior art likewise teaches that any suitable membrane (hydrophobic but can be hydrophilic) can be used to bind nucleic acids** and the membranes claimed by applicants are all either hydrophobic or hydrophilic. Since the prior art teaches that many nucleic acids preferentially bind to hydrophobic surfaces (hence the use of hydrophobic membranes such as polysulfone or polycarbonate, etc. in the cited prior art), the use of membranes which are hydrophobic

> or are coated with hydrophobic substances must be considered obvious to the ordinary skilled artisan. **The choice of the prior art membranes . . . appears to be merely a matter of optimization or design choice which constitutes routine experimentation.** Indeed, given the highly developed nature of the prior art in the area of nucleic acid purification and the well known solutions used in extracting purifying nucleic acids as well as the membranes or filters used to bind or retain nucleic acids (as evidenced by the cited prior art), the ordinary skilled artisan would have been motivated to employ any of the instantly claimed limitations concerning immobilization and washing solutions and nucleic acid binding membranes or filters as any of said solutions and membranes or filters would serve to purify nucleic acids and said compositions and procedures are used for their known and expected results. **Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time of the invention was made, it must be assumed that said skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.**" (pages 8-9, of the Office Action, dated August 8, 2007).

Without ever recognizing the distinguishing steps of binding nucleic acids to one side of a non-siliceous membrane and subsequent elution and retrieval therefrom in Applicants' claimed process in Claim 1 as amended herein, the Examiner has incorrectly stated that the prior art ultrafiltration method of Ogawa teaches the essential steps of Applicants' claimed process. Further, the Examiner broadly states that since the other references describe the use of membranes and reagents in their respective prior art methods for isolating nucleic acids, then the other embodiments of Applicants' claimed invention covered by claims depending from Claim 1 *must* also be obvious. Moreover, the Examiner has adopted the reasoning that a person of ordinary skill in the art would have been motivated to combine Ogawa, Pfister, and Boom (prior Office Action of August 23, 2006) and that in combination with Colpan and Macfarlane there was a reasonable expectation of success. However, the legal standard for rejecting claims as obvious over a combination of references was recently reviewed by the Court of Appeals for the Federal Circuit in *In re Kotzab*, 217 F.3d 1365, 55 USPQ2d 1313 (Fed. Cir. 2000). As the court in *Kotzab* noted:

> "A critical step in analyzing the patentability of claims pursuant to section 103(a) is casting the mind back to the time of invention, to consider the thinking of one of ordinary skill in the art, guided only by the prior art references and the then-accepted wisdom in the field. See *Dembiczak*, 175 F.3d at 999, 50 USPQ2d at 1617. Close adherence to this methodology is especially important in cases where the very ease with which the invention can be understood may prompt one 'to fall victim to the insidious effect of a hindsight syndrome wherein that which only the invention taught is used against its teacher.' *Id.* (quoting *W.L. Gore & Assocs. Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1553, 220 USPQ 303, 313 (Fed.Cir.1983)).

> "Most if not all inventions arise from a combination of old elements. *See In re Rouffett*, 149 F.3d 1350, 1357, 47 USPQ2d 1453, 1457 (Fed.Cir.1998). Thus, every element of a claimed invention may often be found in the prior art. See *Id.* **However, identification in the prior art of each individual part claimed is insufficient to defeat patentability of the whole claimed invention.** See *Id* (emphasis added).

Furthermore, it is well-established that "[b]road conclusory statements standing alone are not evidence" to establish an issue of material fact in law, including for establishing a *prima facie* case of obviousness based on a combination of references. See, *In re Kotzab*, 217 F.3d 1365, 1370, 55 USPQ2d (BNA) 1313, 1317 (Fed. Cir. 2000); *McElmurry v. Arkansas Power & Light Co.*, 99 F.2 1576, 1578, 27 USPQ2d (BNA) 1129, 1131 (Fed. Cir. 1993)). Thus, it is in response to the Examiner's broad conclusory assertions of what is supposedly taught in the prior art methods of Pfister, Boom, Colpan, and Macfarlane that Applicants *must* show on this record that such methods are not equivalent to nor provide a suggestion of Applicants' claimed process and, therefore, there is no rational basis for believing the combination renders Applicants' claims obvious.

The Examiner stated that Pfister was cited to demonstrate the use of standard solutions in lysing cells and immobilizing nucleic acids on filters. However, the disclosure of solutions in Pfister is not a teaching or suggestion of steps of Applicants' claimed process in which nucleic acid is bound to one side of a non-siliceous membrane in the presence of an immobilization buffer and subsequently eluted and removed from that same side of the non-siliceous membrane. As previously documented on this record, Pfister describes a prior art method designed specifically for isolating RNA in which RNA is bound to a specific **silica-gel based fleece** in a centrifugation column and subsequently centrifuged **through the silica fleece for collection on the other side of the fleece**. Applicants' claimed process employs a non-siliceous membrane with two opposing sides, not a silica gel fleece. Applicants' claimed process involves binding nucleic acid molecules to one of the sides of the non-siliceous membrane and subsequent retrieval from that same side, not by passing the eluted nucleic acid through to the other side of the membrane. Thus, the method of Pfister is a distinctly different process from Applicants' claimed invention and there is no motivation to use any solution employed by Pfister to construct and carry out Applicants' claimed process.

Moreover, the prior art method of Pfister is clearly a distinctly different process and chemistry from the prior art ultrafiltration and size exclusion methodologies of Ogawa and Mullis. Thus, a combination of these disparate methods for isolating different types of nucleic acids provides no teaching or suggestion of Applicants' claimed process.

In this Office Action, the Examiner alleges that Applicants' prior response and Dr. Oelmüller's declaration, submitted May 18, 2007, did not address the rationale by the previous Examiner to combine Boom with the other references, i.e., Ogawa, Mullis, and Pfister. This is incorrect. As previously explained in Applicants' response and Dr. Oelmüller's declaration, submitted May 18, 2007, Boom provides an example of the state of the prior art in which it was thought that bound nucleic acids could not be eluted from any non-siliceous membrane. See, e.g., pages 6-7 of Applicants' Response of May 18, 2007, and paragraph 17, of Dr. Oelmüller's declaration, submitted May 18, 2007. In particular, Boom states:

> "[Nucleic acid]-binding filters (see Materials & Methods) can replace SiO_2 in the isolation of nucleic acid according to protocol Y**.
>
> "**Although normally no release of DNA takes place in the low salt buffer . . . this optional problem is set aside by inserting the filter with DNA bound to it in the PCR-reaction mixture instead of eluting the DNA from the filter**." (col. 22, lines 1-15, Boom).

In fact, Boom's prior art teaching of failure to release nucleic acids bound to a non-siliceous membrane is relevant to showing that Applicants' claimed process is nonobvious. As the Supreme Court explained in *Graham v. John Deere*, 383 U.S. 1, 17-18; 86 S. Ct. 684; 15 L. Ed. 2d 545 (1966):

> "Under § 103, the scope and content of the prior art are to be determined; **differences between the prior art and the claims at issue are to be ascertained**; and the level of ordinary skill in the pertinent art resolved. Against this background, the obviousness or nonobviousness of the subject matter is determined. Such secondary considerations as commercial success, **long felt but unsolved needs**, **failure of others**, etc., might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented. As indicia of obviousness or nonobviousness, these inquiries may have relevancy."

As noted above, Boom, which claims priority to 1990, clearly shows failure by others in the prior art for years prior to Applicants' invention to release nucleic acid molecules bound to a non-siliceous membrane. Thus, Boom provides indicia of nonobviousness of Applicants' claimed process. The other embodiments of Boom describe the use a silica particle or silica diatomaceous earth to bind nucleic acid and thus are also clearly not a teaching or suggestion of Applicants' claimed process. Since Boom shows the problem and failure in the prior art, it cannot reasonably be said to contribute to or cure the deficiencies in the combination of the various disparate prior art methods of the combination of references relied on by the Examiner.

The Examiner states that Colpan teaches use of chaotropic agents in an immobilization buffer such as used in embodiments of Applicants' claimed process. The problem with the Examiner's

characterization is that Colpan neither teaches nor suggests Applicants' claimed process. Colpan describes a chromatographic method of isolating nucleic acids in which nucleic acids are adsorbed on a "mineral substrate". Colpan teaches that a mineral substrate useful in the method may be silica gel and materials principally consisting of glass, alumina, zeolites, titanium dioxide, and zirconium dioxide. In addition, every example in Colpan (Examples 1-14) employs a centrifuge chromatographic tube loaded with a mineral substrate to which nucleic acid is bound and then subsequently retrieved by centrifugation or by applying positive pressure to force the nucleic acid *through* and out of the other side of the mineral substrate. Nowhere does Colpan teach or suggest binding nucleic acid to one side of a non-siliceous membrane and subsequent elution and retrieval of the bound nucleic acid from that same side of the non-siliceous membrane. Clearly, the fact that Colpan describes buffers employing chaotropic agents provides no teaching or suggestion of Applicants' claimed process, which also may employ chaotropic agents. Moreover, use of chaotropic agents in buffers for adsorbing nucleic acids to a mineral substrate in combination with Ogawa, Mullis, Pfister, and Boom still does not provide the prior art with Applicants' claimed process because Ogawa does not teach the essential steps of Applicants' claimed process and the other references do not cure the prior art ultrafiltration method of Ogawa of this deficiency.

The Examiner relies on Macfarlane as a teaching of the use of salts of di- or polycarboxylic acids such as oxalic, malonic, citric, and succinic acid in a method of nucleic acid purification (see, e.g., columns 2-4 and Example 3 of Macfarlane). Macfarlane describes the use of a novel cationic quaternary amine surfactant that is employed to lyse cells and complex with the RNA released from the cells. The complex precipitates, and the RNA must be extracted from the complex precipitate, e.g., by a chaotropic salt and optionally phenol extraction or by a formamide buffer, or by solubilizing the surfactant from the precipitate with lithium chloride. Macfarlane describes the use of organic acids in a reaction with a quaternary amine hydroxide to form the cationic novel quaternary amine surfactant salt in which the organic acid salt provides the negative counter-ionic species to the cationic surfactant (see, X^- in Table I, of Example 3, of Macfarlane). Nowhere does Macfarlane teach or suggest Applicants' claimed process in which nucleic acid is immobilized on one side of a non-siliceous membrane by binding the nucleic acid to the one side of the membrane in the presence of an immobilization buffer and wherein the nucleic acid is eluted and retrieved from the same side of the membrane to which it was bound. Clearly, Macfarlane does not cure the deficiency of Ogawa, Mullis, Pfister, Boom, and Colpan to provide the prior art with Applicants' claimed process, let alone any specific embodiment of that process that may employ a particular immobilization buffer containing a salt of a particular di- or polycarboxylic acid.

The above explanations show that the combination of the references cited by the Examiner are at best a collection of examples of various compounds, reagents, and materials used in prior art methods of

isolating nucleic acids that are clearly distinguishable from Applicants' process. However, as noted above, a hindsight reconstruction of a claimed invention from its parts found scattered across unrelated references is clearly not a basis for a *prima facie* case of obviousness because it is unacceptable to use the teachings and guidance of Applicants' own specification to modify the prior art to provide what Applicants now claim. Moreover, Boom in the combination of references actually depicts failures and problems of prior art methods and that there was no reasonable expectation of success in the prior art to formulate and practice Applicants' claimed process, which ultimately has succeeded in solving the very problems and failures that Boom depicts.

Applicants submit that the above explanations clearly show that none of the references teaches or suggests that they be combined to provide Applicants' claimed process or various embodiments thereof. Moreover, even if taken together, the combination of Ogawa, Mullis, Pfister, Boom, Colpan, and Macfarlane provides no more than a collection of disparate prior art methods and existing problems of the prior art, without a discernible teaching, suggestion, or reasonable expectation of success of Applicants' claimed process or any particular embodiment thereof. Accordingly, the combination fails to render Applicants' claims obvious under 35 USC § 103, and the Examiner is respectfully requested to reconsider and withdraw the rejections.

Conclusion

In view of the amendments to the claims and the all of the above comments, Applicants respectfully submit that the Examiner's rejections have been rendered moot or overcome. Accordingly, Applicants respectfully request that the Examiner enter the amendments and withdraw the rejections to pass Claims 1, 3-5, 9-17, 22, 24-31, 33-40, 51, 53-55, 59-64, 69-74, 76-81, and 83-100, as amended herein, to allowance.

Respectfully submitted,

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Date

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